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THE WORK OF THE DIVISION OF IMMUNOLOGY AND ONCOLOGY
OF THE INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY

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- USSR -

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[Following is the translation of an article entitled "O rabote Otdela immunologii i onkologii Instituta epidemiologii i mikrobiologii imeni N. F. Gamelei" (English version above) by L. A. Zil'ber in Vestnik Akademii Meditsinskikh Nauk (Herald of the Academy of Medical Sciences), Vol. 15, No. 7, Moscow, 1960, pages 65-72.]

The work of this Division is dedicated to the study of problems of general immunology, immunology of tumors and their etiology. This brief survey gives only the basic data obtained in the course of the last 2-3 years.

Among the problems of general immunology, a study of the question of a possible differentiation of the immune and the normal globulins was conducted (A. M. Gardash'yan and Z. A. Avenirova). For this purpose gamma-globulins were obtained from the serum of rabbits taken before and after immunization. Gamma globulins of the serum of the same rabbit were compared in a number of experiments. The purity of the preparations was checked electrophoretically. The comparison was done by the method of the cross anaphylaxis reaction with desensitization. These experiments showed an obvious difference in the antigenic structure of the normal and the immune globulin. Thus, the disturbance of the synthesis of the protein caused by the antigen in the process of immunization leads to the formation of globulins which differ in their antigenic structure from the globulins which are synthesized under normal conditions.

Antigenic structure of animal cells was studied in a number of works (G. I. Abelev, Z. A. Avenirova, Z. I. Baidakova, and N. V. Engel'garët). A study of this microsome, the mitochondria and the cell nuclei of the liver of mice has shown that the antigens of mitochondria and microsomes

are very close to each other, but are different from the antigens of the nucleus. The separation of organ-specific antigen of the liver of mice (AO) is of particular interest in the series of these studies. The principle of such separation is based on the fact that AO is bound more stably with an almost insoluble fraction of mitochondria and microsomes (Mm) than the rest of the antigens, and, therefore, can be separated from them.

When the suspension of Mm is destroyed with alkalizing or desoxycholate (0.3 percent), the main bulk of the Mm substance (and the greater part of antigens) changes to a solution, while a considerable part of AO remains bound with the undissolved fragments of Mm which settle in 60 minutes at 100 000 g. After the sediment was washed three times with a 0.3 percent desoxycholate, the antigen was changed into a solution by alkalizing.

A final purification was done with zonal electrophoresis in the density gradient, when AO, which possesses a very low electrophoretic mobility, remained near the point of the zone boundary. At the same time a fraction of non-antigenic lipoproteid which constituted more than 90 percent of the protein of the initial preparation, as well as antigenic admixtures, were separated from it.

The purified AO yielded one clear zone of precipitation in the agar with serums against liver and did not react with antihepatoma serums both in the precipitation reaction in the agar and in the reaction of quantitative precipitation. This antigen possessed a sharply expressed organ specificity in the precipitation reaction and was not evident among the antigens of the Mm of the kidneys, spleen, lungs, and plasma of mice.

The neutralization of the anti-liver serum with a fraction of Mm of the hepatoma and the inclusion of the antigens of the hepatoma into the agar did not hinder the reaction of AO with this serum. AO was found not only in the liver of mice of the C₃HA strain, but also in the liver of mice C₅₇, CC₅₇ and mice having no pedigree. At the present time, the chemical nature of this antigen is being studied.

Along with the antigenic structure of normal animal cells, the antigenic structure of tumor cells was studied. Investigations in the preceding years have established the

presence of antigens in tumor cells which are absent in normal cells (L. A. Zil'ber, 1950, 1957).

The success of these investigations was connected chiefly with the use of the anaphylaxis reaction with desensitization. Lately, the antigenic structure of tumor cells has been studied with the use of the precipitation reaction in the agar on the hepatoma model of C₃HA mice induced by orthoazobenzidine and transplanted to mice of the same strain (G. I. Abelev, Z. A. Avenirova, Z. L. Baidakova, N. V. Engel'gardt).

Unlike the former works of our laboratory, cytoplasmic granules (Mm) were investigated, and not the protein fractions of tissue precipitated at pH 4.5, because our data and the data of other researchers point to antigenic changes of the granules during malignization. Proteins of other fractions were also studied. For a comparative evaluation of the results obtained by different methods, the anaphylaxis reaction with desensitization was also used. Used as antigens were solutions of Mm obtained by alkalizing their suspension to pH 9.0-9.5 with subsequent acidification to pH 7.8-8.2, as well as the extract of granules on 0.14 M NaCl.

Antiserums were prepared by immunizing rabbits against Mm and homogenates of normal liver and of hepatoma. Globulins of these serums were used in the precipitation reactions in the gel which was set up in the usual manner.

In order to expose more clearly the qualitative and quantitative differences of the preparations which were being compared, we applied the following arrangement of the reaction: Homologous serum and antigen are placed opposite one another in the opposite corners of a square. Antigens which are common for the systems which are being compared produce a single spectrum situated between the cavities with the heterologous antigen and the serums. Antigens which are characteristic for only one system produce bands directed along the diagonal of the square formed by the cavities with the antigens and the serums. These bands cd and ef are situated between the cavities with the antigens and the corresponding antiserum and their ends rest on the reservoirs of the heterologous system.

In the arrangement of the experiment described above which was suggested by G. I. Abelev, antigens characteristic

only for one of the systems form bands which differ in their direction from the general spectrum, and antigens and spectrum of two systems are compared here simultaneously. With such a method it was possible to expose qualitative differences between the antigenic structure of the tumor and the normal cells and to find an antigen which is specific for the hepatoma and which is absent in liver. This antigen has also been discovered during the neutralization of anti-hepatoma serums with Mm antigens of liver.

Recently, it became possible to separate a fraction of antibodies to the antigen of transplantable hepatoma which is absent in liver. Antibodies were separated by means of decomposing a precipitate obtained from antihepatoma serum neutralized by the excess of liver antigens during its reaction with the Mm of the hepatoma. The specific antibodies which were obtained facilitate the works on the separation of specific antigen of the hepatoma and the study of its nature.

These studies revealed a simplification of the antigenic structure of the cells during malignization which was described by Weiler (1952-1956) and Seligman, Grabar, Bernard (1955). The photograph shows precipitation lines which indicate that transplantable hepatoma contains an antigen which is absent in liver and at the same time does not have some of the liver antigens. In studying by the same method the nature of antigens which disappear during malignization, it was established that organ-specific antigen of liver (AO) is absent in the Mm of hepatoma, as it was also observed by Weiler in a rat hepatoma. However, anaphylaxis reaction with desensitization revealed an organ-specific antigen in hepatoma extracts containing, apart from Mm, proteins from other fractions (V. I. Gel'shtein). This problem is to be studied further.

Among the studies on the subject of immunology of tumors, we should note the data obtained by V. A. Parnes and D. M. Levina in collaboration with Dr. V. Lacour of the Gustave Roussie Institute in Paris, who worked with us. In agreement with the former data of our staff, V. V. Gordilova and L. V. Shershal'skaya, the antigenic difference of erythrocytes was discovered in leukemia and cancer patients by the precipitation method in the agar on a great amount of material.

The precipitation reaction in the agar was also success-

fully used for the analysis of the antigenic structure of the Rous sarcoma (A. I. Gusev). It was shown that there is a specific precipitating antigen in the Rous sarcoma which is absent in the normal organs of chickens and which differs from the specific antigen of the transplantable chicken sarcoma MX-659. The antigen is a tissue antigen different from the virus of the Rous sarcoma. For the virus of this sarcoma there form antibodies producing a neutralization reaction and not reacting with this tissue antigen.

Serums of cancerous animals were found to be different from the serums of healthy animals in respect to the antigens. This was shown by V. Ya. Shevlyagin when he compared the serums of rabbits having the Brown-Pierce tumors with those of healthy rabbits in the anaphylaxis reaction with desensitization in vitro. However, the specific nature of this reaction needs further study, because cross reactions between the serums of rabbits affected by the Brown-Pearce tumors and the Shope cancer have been observed. These reactions were absent in the serums of rabbits in the presence of papillomas, the Arthus phenomenon and acute inflammation processes. The anaphylaxis reaction with desensitization in vitro is very sensitive. It was successfully used by our colleague, G. T. Patrikeev (1952), for the analysis of organ specificity of animal tissues and was later suggested by Macari (1955) for the detection of a specific antigen in the serum of cancer patients.

Studies conducted in the preceding years (R. M. Radzhikhovskaya, Z. L. Baidakova, O. M. Lezhneva) have shown a possibility of the preservation of the specific antigen in tumor cells lysed with antitumor serums and the ability of these formalinized lysates to immunize against the transplanted tumors and to a certain extent against the primary tumors of animals (experiments with induced sarcomas of inbred mice, naturally developing cancer in mice of the A-strain, Brown-Pearce tumor in rabbits, Rous sarcoma, and others).

Along with the study of antitumor immunity, studies were done on the suppression of resistance in transplanting tumors dependent on the isoantigenic difference (Z. L. Baidakova). The rat sarcoma M-1 transplanted to non-inbred rats and originally induced with benzpyrene (a stock of the L. M. Shaboda Laboratory) could not be successfully transplanted to the Wistar rats, but if these rats were previously treated with the extracts of sarcoma M-1, then the

inoculations were successful and the rats died from this tumor. Such an increased susceptibility continued for only a few days (usually from the 6-7 to 14-15 day after the introduction of the extract of M-1 sarcoma), and then it disappeared. An increased susceptibility was also achieved by the introduction into the Wistar rats of the serum of rats of the same strain which had been intensively immunized with the extracts of M-1 sarcoma.

We have also studied the changes in the antigenic structure of the cells occurring under the influence of ionizing radiation (V. A. Artamonova). In the appropriate experiments, clear antigenic changes were discovered in the liver and the spleen of irradiated rabbits. Independent from us, analogous data were obtained by R. V. Petrov and L. I. Il'yina (1956). In a number of experiments, organs of the same animal (a kidney and part of the liver) were studied before and after irradiation in order to exclude a possible influence of isoantigenic differences. No changes were found in the kidney. In the liver, they were observed in most of the experiments. But sometimes changes were observed after a surgical interference without the action of irradiation. These studies should be repeated on pure-strain animals with the use of various immunological methods.

We tried to use the phenomenon of immunological rapprochement, which was studied for the first time by Hasek (1953), Medawar and their associates (1953), for obtaining serums reacting selectively with the tumor tissue (I. N. Kyukova, R. M. Radzikhovskaya, N. V. Nartsissov, T. I. Biryulina). Rats and rabbits were treated during their embryonic life with the extracts of normal tissues, and, during their adult state, they were immunized with the extracts of tumor tissues. Experiments were conducted with the tumors of animals and human beings. The serums were tested in the complement fixation reaction and in the precipitation reaction in the agar (in the last experiment).

These studies have shown that the introduction of the extracts of various tissues into the embryos of rats and rabbits with a subsequent introduction into them of the same material after birth results in a considerable suppression of the formation of antibodies during the immunization of these animals in their adult state. A suppression of the formation of antibodies was also observed if animals which were tolerant to the normal tissue were immunized with

tumor tissue. However, it was found impossible (with a few exceptions) to differentiate in this manner the extracts of the tumor and normal tissues of human beings.

Different results were obtained in analogous experiments with tumor tissues of animals. With the serums of animals which were tolerant to normal chicken tissues and which were immunized with the extracts of chicken sarcoma, it was possible to differentiate the sarcoma tissue from the tissue of a normal muscle. However, similar experiments with the cancer of mammary gland and hepatoma of mice did not produce results.

The method of artificial immunological tolerance was also used in the study of the heterotransplantation of tumors. A suspension of adenocarcinoma cells of strain mice was introduced into the embryos of the Wistar strain rats. The baby rats which grew from these embryos were inoculated hypodermically with the same tumor when they were one week and two weeks old. In this manner it was possible to obtain in rats an acclimatization of a strain-specific tumor of mice with a subsequent death of one half of the baby rats from this tumor. The tumor was passed into the baby rats three times.

One of the segments of our work is connected with the study of the pathogenic potencies of tumor-producing viruses (I. N. Kryukova, N. V. Nartissova, T. I. Biryulina). In rats infected with the Rous virus, we observed, in the embryonic stage, the development of the hemorrhagic disease with the affection of blood vessels and the formation of large cysts filled at first with a serous and then with a hemorrhagic content. We were unable to separate the virus from these affections, however, G. Ya. Svet-Moldavskiy and A. S. Skorikova succeeded in this (1958), when they made similar observations independent from us. A formalinized virus or a virus neutralized with an antiserum did not cause any pathological phenomena when it was introduced into the embryo of rats, as well as an active virus when introduced into adult rats.

In some rats which recovered after the hemorrhagic disease, tumors developed after long periods of time since the introduction of the virus (about one year). The study of these tumors is in progress. Analogous experiments were done on rabbits. The rous virus introduced into newborn baby rabbits caused a diffused fibromatosis which progres-

sed benignantly and ended with recovery. In none of these cases the virus was separated from the fibrous nodes which had formed.

All these experiments indicate that the same virus can cause different affections, both of the infectious and the neoplastic type, depending on the state and the reaction of the macro-organism. Apparently, the same factors influence to a considerable degree the separability of the virus and its masking. The phenomenon of masking was specially studied on the Shope's virus model adapted to domestic rabbits at the biological station at Sukhumi (V. A. Artamonova). This stock does not become masked when injected into domestic rabbits and can be detected in most of the papillomas, but not in a high titer (not higher than 1:100). During the malignization of the papillomas, the virus becomes completely masked. It was found that a 30 minute contact of the virus in vitro with an extract of a tumor tissue which originated from a papilloma is sufficient for the virus to lose completely its pathogenicity and not to show in the mixture. The virus processed in this manner would even lose its immunization ability, while the virus neutralized by the antibody retained it (Z. A. Postnikova). It was established (V. A. Artamonova) that when cancer tissue is fractionated, the ability to block the virus is connected with the fractions of the tumor tissue which settle at 30 and 50 percent saturation with ammonium sulfate. At present we are attempting to separate the active blocking substance from these fractions.

The etiologival part of our research was dedicated to an electron-microscopic study of virus-like particles detected in tumors by various researchers and attempting to reproduce the cancer disease of human beings in animals.

As it is known, virus-like particles have been detected in many tumors of animals and human beings, whose virus etiology has not been proven. However, similar particles can also be found in normal tissues. Only in very rare cases particles of a peculiar shape were observed (for example, in human leukemia V. A. Parnes observed "caudate" particles). We attempted to obtain serological data for the characteristics of these particles (A. I. Gusev) and to study under an electronmicroscope the agglutination of these particles by specific serums. However, the agglutination titers of antiserums exhausted by the particles from normal tissues were insignificant (1:40) and did not make it possible to make defi-

nite conclusions.

The attempts to transfer cancer disease of human beings to animals are successful, so far, only in respect to human leukemia. By means of introducing various leukemia material taken from leukemia patients or people who died from it into newborn mice of non-leukemia strains, it was possible to cause a transplantable leukemia in mice (V. A. Parnes and V. V. Suntsova). Analogous experiments, but with more detail and based on a greater amount of material, were set up by V. M. Bergol'tz under my direction at the Institute imeni P. A. Gertsen. In his experiments, leukemias in mice were in 34 percent of cases caused by noncellular extracts from human organs affected by leukemia. Formalin and heating at 70° in the course of one hour almost completely destroyed the activity of the factor contained in the extracts which was studied in many other respects. All these studies are in agreement with the data of Magrassi, Rzhiman and Veselyi, and other researchers who were successful in transferring human leukemia to guinea pigs and rats.

At the present time these studies have been considerably broadened (V. A. Parnes, Z. A. Postnikova, I. E. Shustrova, V. Ya. Shevlyagin, I. S. Irlin). They are conducted in the following manner: material taken from a human being is first inoculated in the cultures of a monolayer tissue, and the resistance of these cultures during a part of the experiments is weakened by X-ray irradiation. After a number of subinoculations, the cultures are introduced into the animals which were irradiated and treated with cortisone. Various methods of infecting are used, including the intratracheal method (A. M. Gardash'yan). Studies are also being planned which will take into account the possibility of the presence of a virus component having the form of nucleic acid in human tumors.

We should note the work of the development of inbred strains of mice and rats which was conducted by N. N. Medvedev. A study of the susceptibility to lung tumors of the C₅₇ and CC₅₇ strains, which were developed earlier, is now in progress.

The above studies are only the basic trends of the experimental work of our Division, which can give an idea of our goals and means of their achievement.

Active Member of the AMS USSR
Professor L. A. Zil'ber

PRINCIPLE EXPERIMENTAL WORKS OF THE DIVISION OF
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